Contents

Laboratory of Neurosciences 2

Stanley I. Rapoport, M.D. 5

Mark B. Schapiro, M.D. 8

Barry Horwitz, Ph.D. 11

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The Laboratory of Neurosciences (LNS) was established in 1978 at the Gerontology Research Center in Baltimore. In 1984, the LNS moved to the Clinical Center on the Bethesda Campus, where clinical research could be performed using outpatient and inpatient facilities and available brain imaging capabilities. The LNS currently is divided into two sections that were formed in 1982: a basic research Section on Cerebral Physiology and Metabolism (Stanley I. Rapoport, M.D.) and a clinical research Section on Brain Aging and Dementia (Mark B. Schapiro, M.D., Chief).

The basic and clinical sections of the LNS interact to study brain function, metabolism and structure with regard to aging and disease, including Alzheimer's disease, Down's syndrome (which inevitably leads to Alzheimer-like neurodegeneration) and hypertension. Clinical protocols use positron emission tomography (PET) to measure regional brain metabolism and blood flow, functional (fMRI) and structural magnetic resonance imaging to measure brain blood flow and volumes of brain structures, magnetic resonance spectroscopy to measure brain metabolites such as *myo*-inositol and phosphorus compounds, and neuropsychological and behavioral assessment to evaluate normal cognition and dementia. Protocols are designed to quantify reorganization of brain functional networks, brain atrophy and changes in brain metabolites in relation to aging and disease, to identify and characterize the pre-clinical and clinical phases of Alzheimer's disease, and to evaluate pharmacotherapeutic efficacy in aging and Alzheimer's disease. Activation studies involving PET and fMRI use cognitively task or passive stimulation that are varied parametrically with regard to stimulus intensity and/or difficulty, as "stress tests" of brain function. The effects of physostigmine (cholinergic antagonist) and other modulators of neurotransmission on performance and brain responses are evaluated in tests. Studies are related to genetic markers (apolipoprotein E alleles) and post-mortem brain studies. An hypothesis being tested is that Alzheimer's degeneration reflects progressive synaptic and related metabolic-flow dysfunction, particularly in brain association areas, and that the first stage of this dysfunction is potentially reversible whereas the later stage is not. We currently are

examining central cholinergic mechanisms using PET in relation to this hypothesis, using labeled ligands of brain cholinergic receptors, muscarinic agonists and antagonists (arecoline and physostigmine), and labeled arachidonic acid to study cholinergic signal transduction.

In the basic section, post-mortem brain from clinical control and Alzheimer's patients is subjected to histochemical, molecular, immunocytochemical and enzymatic techniques to examine the basis of reduced glucose metabolism and blood flow in life. Evidence for reduced expression of genes coding for subunits of mitochondrial oxidative-phosphorylation enzymes supports staging of metabolic dysfunction into potentially reversible and irreversible steps. Additionally, animal models for Down syndrome (trisomy 21)-- trisomy 16 and trisomy 65DN mice (the 16th mouse chromosome corresponds to human chromosome 21)-- are developed to understand mental retardation in that disorder. Abnormal ionic currents and ionic channel densities in cultured neurons from these models, and accumulation of *myo*-inositol in their brain, suggest specific defects in signal transduction which may contribute to mental retardation. Thirdly, an experimental *in vivo* model has been developed in the LNS to quantify brain membrane remodeling and signal transduction involving phospholipids, using radiolabeled long chain fatty acids. The model is elucidating changes in phospholipid metabolism during recovery from cerebral ischemia, in an animal model for Alzheimer's disease (nucleus basalis lesion), with regard to the mechanism of action of lithium, and in relation to central drug action. It recently has been extended to clinical PET studies (see above). Finally, an analytical laboratory quantifies concentrations of drugs and their metabolites in clinical studies and related basic experiments.

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Recent Publications:

Hatanpää K, et al. *Ann Neurol* 1996; 40: 411-420.

Rapoport SI, *Lipids* 1996; 31: S97-S101.

Rapoport SI, et al. *Neurodegeneration* 1996; 5: 473-476.

Stoll J, et al. *Int J Dev Neurosci* 1996; 14: 749-760.

Biography: Dr. Rapoport received his M.D. from Harvard Medical School in 1959, interned in Medicine at Bellevue Hospital, New York, from 1959-1960, and received post-doctoral research training at the Department of Physiology, University of Uppsala, Sweden, and at the Laboratory of Neurophysiology, National Institute of Mental Health (NIMH). He was appointed as a tenured scientist at NIMH in 1968, and in 1978 Chief of the Laboratory of Neurosciences, NIA. He is a Fellow of the American College of Neuropsychopharmacology, the American Academy of Neurology and the Gerontological Society of America.

The Section on Cerebral Physiology and Metabolism studies basic aspects of brain function and metabolism, particularly in the following areas:

Markers of Oxidative Phosphorylation (OXPHOS) Within Brain Mitochondria Suggest Two Stages of Functional Failure in Alzheimer Disease: *In vivo* brain imaging in Alzheimer's disease (AD) patients using positron emission tomography (PET) demonstrated reductions in cerebral glucose consumption and blood flow, progressing with dementia severity. Post-mortem brain studies showed that the reductions corresponded to reduced regional brain activity of cytochrome oxidase (rate limiting enzyme for mitochondrial oxidative phosphorylation, OXPHOS). Furthermore, mRNA levels for mitochondria (mt)-encoded subunits COX I-III and for nuclear-encoded subunit COXIV were reduced, whereas mt-encoded 12S rRNA and total mtDNA were unchanged. A similar pattern of reduction (down regulation) has been reported in monkey lateral geniculate nucleus following visual deprivation, and can be reversed following restoration of vision. We then quantified COX III mRNA by *in situ* hybridization in individual pyramidal AD neurons in relation to cytoplasmic density of neurofibrillary tangles. Compared with tangle-free neurons from control brains, tangle-free AD neurons showed reduced COX III mRNA but no change in mt-encoded 12s rRNA or polyadenylated mRNA. COX III mRNA fell in relation to tangle content until tangles filled more than 50% of the neuronal cytoplasm, when

non-OXPHOS RNA levels also were reduced. We hypothesize that an early event in AD is dysfunction of synapses (where most energy is consumed), leading to reduced neuronal energy demand and potentially reversible down-regulation of OXPHOS. This is consistent with our evidence that the AD brain can be fully activated early in disease. In later stages of disease, interference with mitochondrial delivery to distant dendrites by cytoplasmic neurofibrillary tangles likely causes irreversible neuronal loss. In the next year, we intend to evaluate our hypothesis by identifying the factors that couple neuronal activity to expression of OXPHOS in the brain and how they may be affected in AD, and by quantitatively relating markers of early synaptic dysfunction in AD to OXPHOS gene expression.

Mouse Models for Mental Retardation in Down Syndrome: Down syndrome (DS, trisomy 21) is the major known genetic cause of mental retardation (1/1000 births) and leads inevitably to AD neuropathology after age 35. Our section uses molecular and electrophysiological techniques to examine functional and metabolic deficits in animal models of DS: mouse trisomy 16 (which dies in utero) and partial trisomy (65DN) (which survives and shows learning deficits), where the 16th mouse chromosome contains genes found on human chromosome 21. Hippocampal fetal Ts16 neurons in primary culture showed abnormal action potentials and abnormal ionic currents. A reduced inward Na current corresponded to reduced membrane Na channel density. Normal levels of mRNA's coding for Na channel subunits suggested post-transcriptional defects in Na channel expression. Additionally, *myo*-inositol was elevated by 50% in the Ts 65DN mouse brain and in cerebrospinal fluid from DS subjects, possibly reflecting localization of a myoinositol transporter gene on mouse chromosome 16 and human chromosome 21. Excess *myo*-inositol may interfere with the phosphatidylinositide cycle involving phospholipase C. Thus, retardation in DS may arise from abnormal signal transduction arising from a number of causes. Future research will study the mechanisms of the observed changes.

***In vivo* Imaging of Brain Phospholipid Metabolism:** Phospholipids are major constituents of cell membranes and participate in neuroplastic remodeling and signal transduction. We developed in rats an *in vivo* method and model to localize and quantify brain phospholipid metabolism, and turnover of fatty acids within specific sites of brain phospholipids. A radiolabeled long chain fatty acid (unsaturated arachidonate or docosahexaenoate, saturated palmitate) is injected intravenously and its rate of incorporation into brain is measured using quantitative autoradiography and chemical analysis. With this model, we showed in rats that recovery from the massive release of fatty acids due to

cerebral ischemia is promoted by selective reincorporation of arachidonic acid (precursor for prostaglandins and prostacyclins) into brain phospholipids. Lithium, used clinically to treat manic depressive disorder, reduces arachidonate turnover by some 80% without affecting turnover of docosahexaenoate and palmitate, and thus likely acts at phospholipase A2. Additionally, 11C-labeled fatty acids were synthesized in collaboration with the PET Department at NIH and were used to image phospholipid metabolism of monkey brain with PET (tracer uptake was independent of blood flow) and to initiate a clinical PET protocol on healthy controls and patients with AD. In the coming year, we plan extend this protocol to evaluate cholinergically-mediated signal transduction in AD patients, to develop a fluorescent method to measure regional phospholipase A2 activity in brain slices in relation to local fatty acid metabolism, and to phospholipase A2-mediated signal transduction involving the brain dopaminergic system.

Collaborators: Charles Epstein, Department of Pediatrics; University of San Francisco School of Medicine; William Eckelman, PET Department, CC, NIH; Nancy Lane, University of Cambridge, UK; Scott Eleff, Department of Anesthesiology, Johns Hopkins School of Medicine; Greg Gillen, National Institute of Standards and Technology; Jeff Alger, Department Radiological Sciences, UCLA School Medicine; Alfred Yergey, Laboratory of Cellular and Molecular Biophysics, NICHD.

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Recent Publications:

Dani A, et al. *NeuroReport* 1996; 7: 2933-2936.

Pietrini P, et al. *Am J Psychiatry* 1997; 154: 1063-1069.

Krasuski JS, et al. *Biological Psychiatry* 1998; 43: 60-68.

Alexander GE, et al. *NeuroReport* 1997; 8: 1835-1840.

Biography: Dr. Schapiro received his M.D. from the University of Tennessee in 1976. After completing residencies in Pediatrics and Neurology, he served in the Laboratory of Neurosciences in 1983 as a Medical Staff Fellow and, later, a Senior Staff Fellow before becoming Chief, Section on Brain Aging and Dementia in 1990.

Neurodevelopmental Disorders: Down Syndrome: One goal of the Section on Brain Aging and Dementia is to understand the genetic determinants of brain aging using *in vivo* brain imaging in relation to mental retardation and vulnerability to Alzheimer's disease (AD). Our work has focused on Down syndrome (DS), a disorder in which an overexpression of different genes on chromosome 21 leads to mental retardation and dementia. In studies designed to examine the hypothesis that defective signal transduction causes mental retardation in DS, we showed a 50% increase in *myo*-inositol (a molecule involved in signal transduction) that is related to cognition, suggesting a gene dose effect of the extra chromosome 21 on which the human sodium/*myo*-inositol cotransporter gene is located. We will follow up these findings with a study in DS adults who have received lithium, which disrupts *myo*-inositol recycling. Additionally, we reported a 50% increase in choline (a precursor of membrane phospholipids and the neurotransmitter acetylcholine), consistent with a gene dose effect and suggesting that a locus on chromosome 21 is responsible for choline homeostasis.

Genetics and the Preclinical Stages of Alzheimer's Disease: Another goal of the Section is to develop techniques for the early diagnosis of AD, since AD may be more reversible in the earlier rather than the later stages of disease. To develop such techniques, we studied older DS adults who develop AD after age 40 years. We showed that the dementia syndrome in DS is phenotypically similar to AD and that the dementia syndrome can occur without the mental retardation, suggesting that only a portion of the

brain cells need to be trisomic for chromosome 21 genes for the development of dementia. We currently are testing this hypothesis with an *in situ* hybridization study of the brain to quantify the location and degree of trisomic cells.

Because there is variation in age of onset of dementia and survival in DS, we studied the influence of other genetic factors. We showed that two genetic factors (amyloid and apolipoprotein E4) can interact to accelerate the development of AD in DS. We further showed that language function, which is relatively preserved in the preclinical stages of AD in DS, is related to APOE genotype. We are continuing these studies to understand how changes in brain metabolism are related to APOE genotype.

Longitudinal evaluation of cognition, brain structure, and resting metabolism showed 2 phases of decline in older DS subjects. There was first a stable phase of at least 7 years preceding onset of dementia with no alteration in brain function or structure. This phase was followed by a linear decline in cognition, structure and function coincident with onset of dementia, suggesting a fundamental change in brain physiology with onset of dementia.

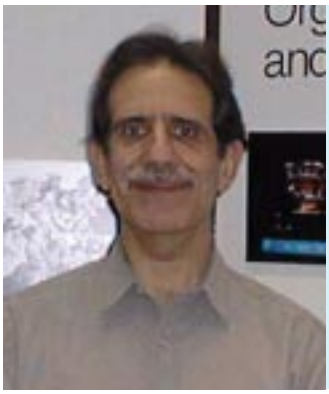
Given the stability of the first phase, we developed methods for the preclinical detection of AD. We showed that subjects in the preclinical phases of AD have medial temporal lobe atrophy which selectively correlates with memory performance, showing that the medial temporal lobe is the earliest site of AD. We plan to apply a discriminant analysis method to medial temporal lobe volumes to identify subjects at risk for AD on the basis of other genetic markers.

We also have identified brain changes in the preclinical stages of AD using PET scanning. By examining brain metabolism while the subjects rest with a discriminant analysis, we were able to identify subjects at risk as having AD. Further, an activation PET scanning procedure showed abnormalities in regions known to be affected in AD in DS subjects in the preclinical stages of AD. These findings show that analysis of PET metabolic data can be used for early detection of AD in individuals at risk. We plan to continue our studies of early diagnosis in DS and in other genetic models of AD using PET scanning with other activation paradigms developed in our Laboratory. We further plan to refine techniques that will quantitate an individual’s risk of AD using resting and activation PET data. Finally, based on these techniques, we plan to initiate therapy early and evaluate the effectiveness of treatment with the above techniques.

Mechanisms of Disease: A final goal of the Section is to conduct experiments on the pathophysiology of AD in order to understand the cause of the treatment failure. The implications of loss of synapses (which are necessary for effective neurotransmitter replacement) in AD are being studied with PET scanning. We recently combined PET with psychophysical stimulation and showed that AD patients in the early to middle stages of disease retain the capability to respond to stimulation despite baseline resting hypometabolism. However, in the later stages of disease, there is a loss of the capability to respond to stimulation up to the point where no response occurs. Such findings emphasized that different stages of AD may respond differently to therapies. PET is now being combined with cognitive and pharmacologic stimulation in order to further study the efficacy of neurotransmission and compensatory mechanisms as a function of disease severity. Currently, cognitive stimulation paradigms are being used that require active participation (ie, working memory, and attention) or require only passive viewing (ie, viewing a movie, visual textures, flashing lights). Further, drugs are being used to modulate the degree of activation from cognitive stimulation. Additionally, to understand the postmortem findings of loss of presynaptic M2 cholinergic receptors but not M1 postsynaptic receptors, a new ligand to examine specific muscarinic receptor densities has been developed in collaboration with the Nuclear Medicine Department, Clinical Center.

We are also examining intracellular signal transduction as a result of postmortem findings suggesting that it is compromised in AD and results in failure of neurotransmission. Metabolites of phospholipid metabolism that are involved in signal transduction (i.e., *myo*-inositol, choline) are being measured in AD and older DS adults with ^{1}H magnetic resonance spectroscopy. Additionally, an *in vivo* brain imaging method to study receptor mediated stimulation of phospholipid metabolism through activation of phospholipase A2 is being conducted. This method will allow quantitation of the regionally selective turnover of the fatty acid arachidonate in the sn-2 position of phospholipids at rest and during stimulation.

Collaborators: Umesha Shetty, LNS; Melvin Ball, M.D. and Geoffrey Murdoch, M.D., Ph.D., Oregon Health Sciences University; Ann Saunders, Ph.D. and Allen Roses, M.D., Duke University Medical Center; Katherine Sanford, Ph.D., NCI; Jay Robbins, M.D., NCI; Neill Graff-Radford, M.D., Mayo Clinic Jacksonville.



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Recent Publications:

McIntosh AR, et al. *Cerebral Cortex* 1996; 6: 571-584.

Rumsey JM, et al. *Brain* 1997; 120: 739-759.

Biography: Dr. Horwitz was trained as a theoretical physicist at the University of Pennslyvania, where he received his Ph.D. He taught physics at King's College (Wilkes-Barre, PA), Vassar College, and Texas Women's University before moving to the Laboratory of Neurosciences as a Senior Staff Fellow in 1982. He became a tenured Research Mathematician in 1988. His major research interests focus on using functional brain imaging data and neural modeling methods to understand how the brain performs specific cognitive tasks, and how these are altered by aging and disease.

Network Modeling of Neuroimaging Data: The major goal of the Brain Imaging and Computers Unit is to develop methods to understand how the brain constructs networks of interacting regions (i.e., neural networks) to perform cognitive and sensorimotor tasks. In particular, a major focus concerns how these networks are altered during healthy aging, and in brain diseases such as dementia. These issues are addressed by combining computational neuroscience techniques with functional neuroimaging data, obtained using positron emission tomography (PET) or functional magnetic resonance imaging (fMRI). The network analysis methods developed in this laboratory allow us to evaluate how brain operations differ between tasks, and between normal and patient populations. With respect to disease, this research allows us to ascertain which networks are dysfunctional.

A second major area of investigation concerns using statistical techniques applied to neuroimaging data to discern differences between individual patients and healthy subjects. The goals are early detection of disease, assessing therapeutic interventions, and differentiation of patient subgroups. In particular, a major thrust is the development of cognitive "stress" tests that would result in different functional neuroimaging patterns (as evidenced by altered neural network behavior) between patients with very early dementia and healthy controls. Looking for subtle

deviations from normality among the brain regional interrelationships may provide the extra dimension needed to identify abnormalities in the scans of single individuals before the appearance of clinical abnormalities.

The method of structural equation modeling is used for constructing systems-level network models from an experimentally obtained interregional correlation matrix. In the structural equation models, one combines two sets of data: (1) the known neuroanatomical connections between brain areas, and (2) the interregional correlations between these regions. One attempts to calculate the functional strength of each anatomical path (called path coefficients or functional couplings), which represents the magnitude of the influence of each directional path. The best-fit of the combination of the anatomical network and interregional correlations creates a functional network for each group/condition. The functional networks can be compared between tasks or groups to identify task-specific or group-specific functional interactions within the same anatomical network.

Structural equation modeling was applied to data obtained during a PET study of object vision (using face matching as the task) versus spatial vision in young subjects. It was found that the functional network for the right cerebral hemisphere showed dominant path influences that included ventral occipitotemporal, anterior temporal and frontal areas during the object vision task, whereas the dominant influences in the spatial vision task included dorsal occipital, parietal and frontal areas. Recently, we extended the analysis of the face matching task to healthy old subjects and to patients with mild dementia of the Alzheimer type (DAT) and found that the old healthy controls had strong functional linkages in the same ventral network discussed above for young subjects. The functional linkages in the DAT group involving the ventral frontal area with posterior extrastriate regions were markedly reduced. However, frontal areas showed more extensive positive correlations with other frontal areas in the DAT patients than in controls. These results suggest that mildly demented DAT patients, who are able to perform the face matching task with the same accuracy as the controls, are utilizing different neural circuits than do controls, emphasizing the critical role played by neural plasticity in early DAT.

In the last year, this method has been used to delineate the functional networks involved in a delayed match-to-sample object vision task, and in two single word reading tasks. In the latter, in collaboration with Judith Rumsey, we found that the left angular gyrus plays a critical role in single word reading in normal subjects, but is functionally disconnected from other network components in subjects with developmental dyslexia.

In order to understand the relationship between what is observed in functional neuroimaging studies and the underlying neural dynamics, a large-scale computer model of neuronal dynamics that performs an object-matching task similar to those designed for PET studies was implemented. The model is composed of elements that correspond to neuronal assemblies in cerebral cortex, and contain different elements that are based on types identified by electrophysiological recordings from monkeys as they perform similar tasks. It includes an “active” memory network involving the occipitotemporal visual pathway and a frontal circuit, and is capable of performing a match-to-sample task in which a response is made if the second stimulus matches the first. A PET study is simulated by presenting pairs of stimuli to an area of the model that represents the lateral geniculate nucleus. rCBF data are computed from the model as it performs the tasks by integrating synaptic activity within the different areas. Simulated rCBF data similar to that found in PET delayed match to sample visual tasks was obtained, as were the correct neuronal dynamics in each brain region.

In the coming year, we plan to fully delineate the functional networks associated with reading and naming. The effect of cholinergic agonists on the delayed match to sample systems level network also will be investigated. The large-scale neural network will be expanded to include more brain regions so that more complex tasks can be investigated. Because one can include specific types of pathologies in simulated “brains”, explicit tests of hypotheses concerning how neural networks are altered by diseases such as Alzheimer’s dementia can be assessed.

Collaborators: Cheryl Grady, Ph.D., Rotman Institute (Toronto); Judith Rumsey, Ph.D., National Institute of Mental Health; Marie-Pierre Deiber, Ph.D., Robert Weeks, M.D. and Mark Hallett, M.D., National Institute of Neurological Diseases and Stroke; Susan Resnick, Ph.D., Laboratory of Personality and Cognition, National Institute on Aging; Malle Tagamets, National Institute of Diabetes and Digestive and Kidney Diseases; Hans Mueller-Gaertner, M.D. and Bernd Krause, M.D., University of Dusseldorf; Adrian Owen, Ph.D. and Trevor Robbins, Ph.D., Cambridge University.